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PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Applicant:	GRAFF Jeremy Richard	Group Art Unit:	4173
Serial No.:	10/573,632	Examiner:	Benjamin J. Packard
Application Date:	October 8, 2004	Conf No.:	9016
US Nat'l Entry Date (if applicable):	March 28, 2006		
For:	BISINDOLYL MALEIMIDES USEFUL FOR TREATING PROSTATE CANCER AND AKT-MEDIATED DISEASES		
Docket No.:	X16348		

DECLARATION UNDER 37 C.F.R. § 1.132

Assistant Commissioner for Patents

Washington, D. C. 20231

Sir:

I, Dr. Jeremy Graff, declare that:

I hold the degree of Ph.D. in Microbiology and Immunology. I received my degree in 1994 from the Department of Microbiology and Immunology at the University of Kentucky. My Ph.D. thesis was entitled "Messenger RNA translation and malignancy: The mRNA cap-binding protein, eIF-4E, as an integral component of malignancy in cloned rat embryo fibroblasts."

I have been employed since 1998 by Eli Lilly and Company as a Research Scientist in Cancer Research. I am now a Research Advisor in the Cancer Growth and Translational Genetics Group within the Cancer discovery group in Lilly Research Laboratories.

I have authored or co-authored 41 research papers published in scientific journals.

I further declare that the following routine experiments demonstrate activity of the compounds of Formula I against human tumor cell lines which include those derived from Cancers of the prostate, and colon as well as glioblastoma and B cell lymphoma, and that these experiments were carried out under my supervision and control.

Experimental Procedures

All experiments conducted in the *in vitro* and *in vivo* efficacy assessment of the compounds of Formula I followed commonly utilized methodology and protocols as described (Graff et al., *Cancer Research,* 65(16):7462-9. 2005).

Results

As used herein, reference compound LY436881 (also known as enzastaurin) refers to the mono-hydrochloride salt form of LY317615 shown on page 10, lines 1-4 of the specification. Similarly, LY482403 refers to Compound 1 described on p. 10, lines 5 of the specification, representing the compounds of Formula 1 in the claimed methods of use.
As shown in Table 1, results from the UBI kinase profiler analysis demonstrate that both LY436881 and LSN492403 exhibit PKC beta activity below the 1μM level (.2μM for LY436881 versus .02 μM for LSN492403). However, only LSN492403 exhibits AKT-3/PKBgamma activity below the 1μM level (1.24 μM for LY436881 versus .15 μM for LSN492403). Similarly, for p70S6K (another kinase in the AKT pathway), activity is 1.85 μM for LY436881 versus .4 μM for LSN492403.
The AKT pathway is known to regulate cell survival and, accordingly, its inhibition induces programmed cell death (apoptosis). Figure 1 demonstrates that LSN492403 induces apoptosis in multiple, cultured human cancer cell lines derived from androgen-dependent and androgen dependent prostate cancers. In contrast, Enzastaurin induced apoptosis only in androgen-dependent LNCaP cells. Figure 2 further demonstrates that LSN492403 induces apoptosis in cultured human cancer cell lines derived from colon cancers, as well as from glioblastomas and B cell lymphomas. Enzastaurin did not demonstrate substantial apoptosis in these cell lines.

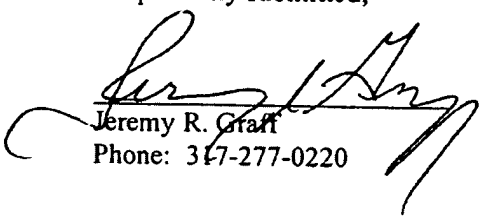
Collectively, these data indicate that relative to enzastaurin, LSN4924903 robustly suppresses signaling through the AKT pathway and induces programmed cell death/ apoptosis in multiple human cancer cell types.

Conclusion

The results of the foregoing experiments demonstrate that relative to enzastaurin, the compounds of Formula I have substantial activity in the AKT pathway and induce apoptosis in human tumor cell lines derived from prostate and colon cancers, as well as glioblastomas and B cell lymphomas.

I further declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both (18 U.S.C. 1001), and may jeopardize the validity of this application or any patent issuing thereon.

Respectfully submitted,



Jeremy R. Graff
Phone: 317-277-0220

Eli Lilly and Company
Patent Division/
P.O. Box 6288
Indianapolis, Indiana 46206-6288
July 3, 2008

Attachments (1):

TABLE 1

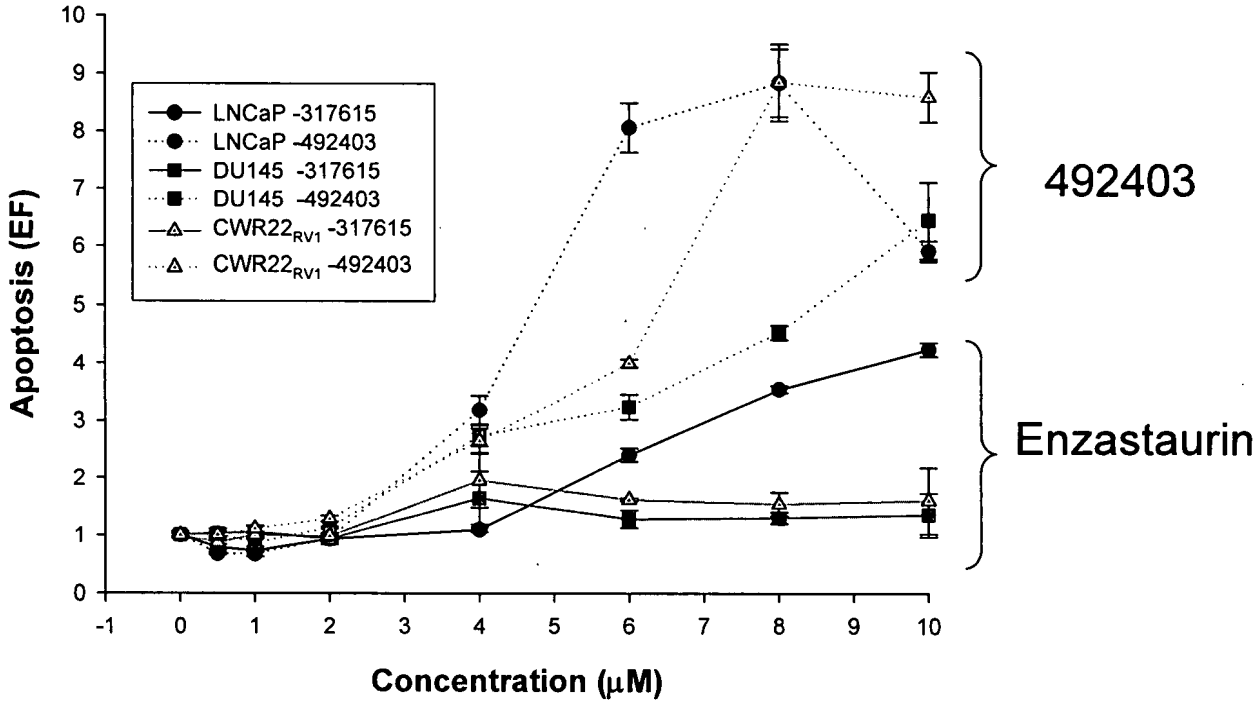
Figure 1

Figure 2

TABLE 1: Kinase inhibition (IC50 data in μM)- UBI kinase profiler assay

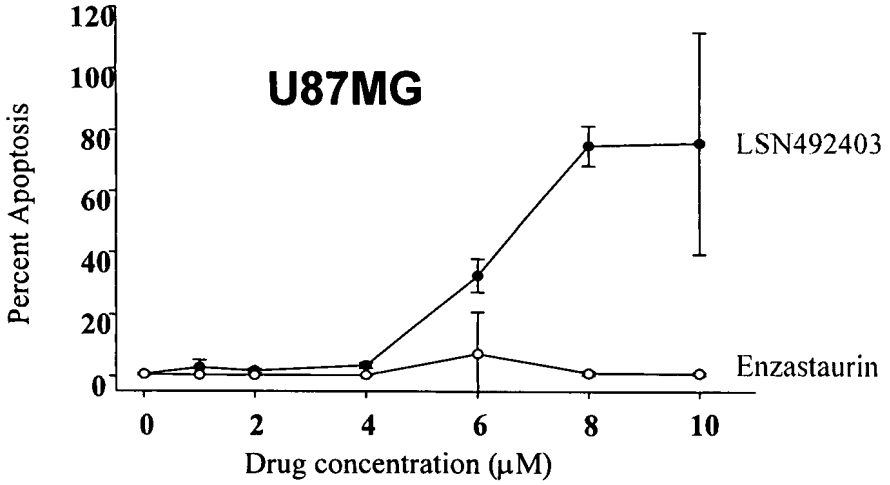
Kinase	**LY436881**	**LY492403**
PKCbeta1	0.15uM	0.01uM
PKCbetaII	0.19uM	0.02uM
PKBgamma(AKT-3)	1.24uM	0.15uM
p70S6K	1.85uM	0.40uM
P90rsk	0.03uM	0.01uM

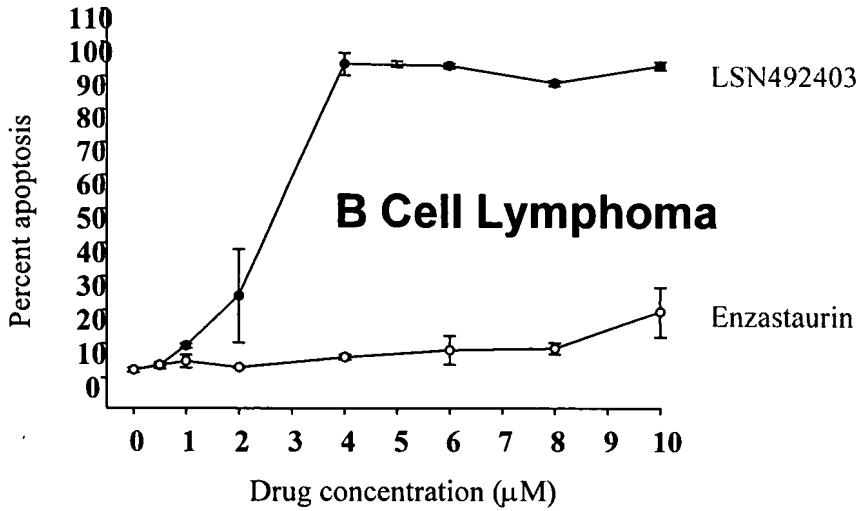
FIGURE 1: Forty-eight Hour Apoptosis in Prostate Cancer Cell Lines

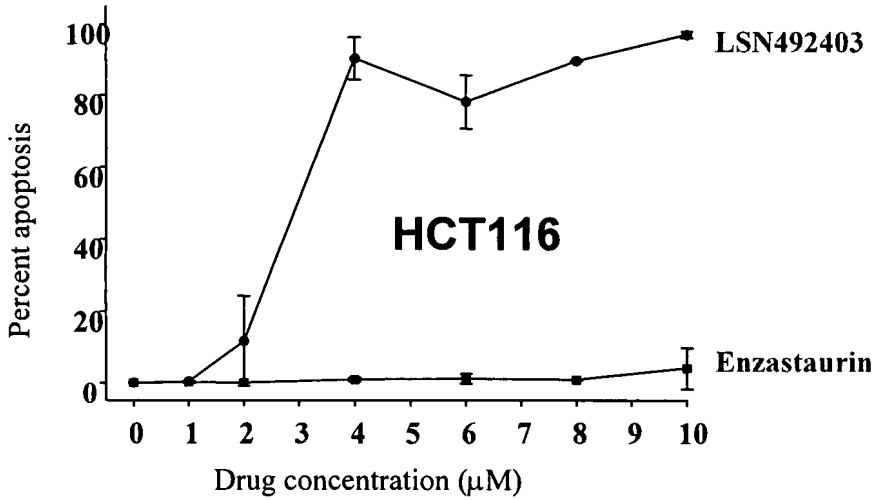


LY492403 induces apoptosis in the androgen-dependent LNCaP, as well as the androgen-independent Du145 and CWR22Rv1 cells. LY436881 can also induce apoptosis but only in the androgen-dependent LNCaP cells. Apoptosis was measured by oligonucleosomal fragmentation (Roche cell death detection ELISA) and expressed as an enrichment factor. An EF > 2 signals apoptosis.

FIGURE 2: LY492403 induces apoptosis in U87MG glioblastoma cells, HCT116 colon cancer cells, and Diffuse Large B Cell Lymphoma cells







Apoptosis was assessed by TUNEL staining 48 hours post drug treatment. Cells were cultured in complete medium supplemented with 10% FBS. Drug concentrations are indicated.